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Bioelectrocatalytic generation of directly readable code: harnessing cathodic current for long-term information relay†

Guinevere Strack,[ad] Heather R. Luckarift,*[ab] Robert Nichols,[ac] Kristofor Cozart,[ac] Evgeny Katz[d] and Glenn R. Johnson*[a]

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Here we present an exceptionally stable bioelectrocatalytic architecture for electrocatalytic oxygen reduction using a carbon nanotube electrode as the electron donor and a fungal enzyme as electrocatalyst. Controlling oxygen content in the electrolyte enables generation of a directly readable barcode from monitoring the enzyme response.

Digital paradigms have been applied to chemical and biochemical systems with a variety of outcomes, for example, information processing,[1–7] encryption,[8,9] and security systems.[10,11] Another form of chemical/biochemical-based digital information conveyance is the unconventional barcode.[12–21] Several bar codes serving as medical diagnostic models or multiplexed bioanalytical assays have been developed with applications ranging from the detection of multiple proteins[13,17] to single molecules of DNA.[20] In addition, unconventional barcodes serving as encryption tags were demonstrated with metallic nanowires[14,15,18] and quantum dots.[12,13] Although not all of the given examples are considered digital sequences, the commonality is the directly readable information providing medically relevant diagnostic or other encoded information within a single-use report. An unexplored approach to the unconventional barcode is the generation of continuous digital information though a bioelectronic interface. In this concept, the biocatalytic electrode can control current density in a self-powered device and/or decipher received signals from a variety of chemical/biochemical sources with an intrinsic write–rewrite function.

Practical application of enzyme-based electronics and fuel cells requires that the biocatalysts withstand environmental fluctuations and continually catalyze redox reactions.[22,23] Enzyme-based Boolean logic has been demonstrated to control biofuel cells by coupling signal-responsive materials and soluble redox mediators for electron transfer.[24–26] To render self-powered bioelectronic devices fit for sustained built-in information processing under continuous flow, the present work uses a laccase-functionalized architecture that responds directly to a continuous *input* chemical signal (*i.e.*, dissolved O_2) and generates an amperometric *output* response. Refined control of the input conditions extends the utility of the platform to generate commonly recognized code.

For optimal electrode fabrication, hierarchically ordered carbon nanomaterials that are scalable and manufacturable provide a clear advantage for subsequent development. In this study, electrodes were composed entirely of a multiwall carbon nanotube (CNT) material commonly referred to as "buckypaper" (CNT-BP) providing a flexible and highly electrically conductive (51.2 $S \cdot cm^{-2}$) surface area for enzyme immobilization. A heterobifunctional cross-linker, 1-pyrenebutanoic acid, succinimidyl ester (PBSE), was used to effectively link laccase to CNT-BP. The connection occurs by virtue of a succinimidyl residue that provides covalent bonding to protein amines, and an aromatic pyrenyl moiety that interacts with CNT through π–π stacking.[27,28] The PBSE-modified CNT-BP alone shows capacitive current and an open circuit potential (OCP) of 0.22 V, but lacks any Faradaic current within the given potential range.

CNT-BP electrodes functionalized with laccase reduce O_2 at an onset potential of 0.635 ± 0.016 V; $n = 6$, and an OCP of 0.627 ± 0.01 V; $n = 5$ (*vs.* Ag/AgCl), in good agreement with previous reports showing oxygen reduction with direct electron transfer (DET) processes[29,30] (Fig. 1, inset). Measurements of O_2-free electrolytes by cyclic voltammetry (CV) lack the catalytic cathodic peak. The quasi-reversible process at −0.029 V was likely the oxidation and reduction of copper-containing active centers in the enzyme, no such response was observed in the absence of laccase.[29,30] Aerobic potentiostatic and galvanostatic polarization data demonstrated current densities in excess of 100 $\mu A \cdot cm^{-2}$ indicating that the CNT-BP provides an excellent support for laccase-catalyzed O_2 reduction (Fig. 1). Potentiostatic polarization data in N_2-saturated electrolyte exhibited minimal current density, as expected in the absence of O_2.

Sustained DET between enzymes and electrodes is influenced by several factors, in particular enzyme stability and effective electron transfer from electron donor to the terminal

[a] *Air Force Research Laboratory, Airbase Sciences Division, Tyndall Air Force Base, Florida, 32403, USA. E-mail: Glenn.Johnson@tyndall.af.mil*
[b] *Universal Technology Corporation, 1270 N. Fairfield Road, Dayton, Ohio, 45432, USA. E-mail: Heather.Luckarift.ctr@tyndall.af.mil*
[c] *Applied Research Associates, 430 West 5th St, Panama City, Florida, 32401, USA*
[d] *Department of Chemistry and Biomolecular Science, Clarkson University, Potsdam, New York, 13699, USA*

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14. ABSTRACT
Here we present an exceptionally stable bioelectrocatalytic architecture for electrocatalytic oxygen reduction using a carbon nanotube electrode as the electron donor and a fungal enzyme as electrocatalyst. Controlling oxygen content in the electrolyte enables generation of a directly readable barcode from monitoring the enzyme response.

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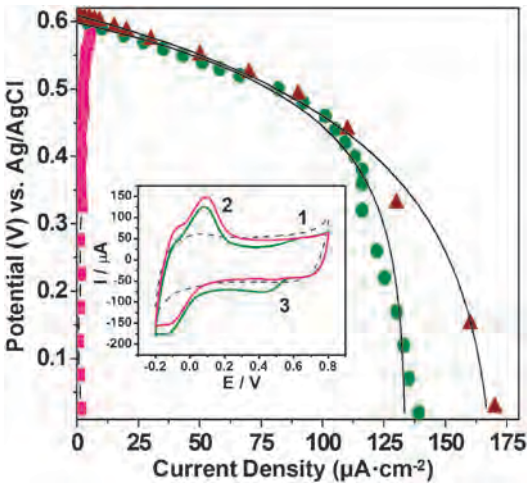

Fig. 1 Oxygen reduction catalyzed by laccase tethered to CNT-BP. Potentiostatic ● and galvanostatic ▲ polarization curves in oxygen-saturated electrolyte and potentiostatic ■ under nitrogen-saturated electrolyte. (Inset) Cyclic voltammetry of the PBSE-modified CNT-BP electrode in nitrogen without the enzyme (1, dashed line), with immobilized laccase in nitrogen (2) and (3) oxygen saturated electrolyte, scan rate = 10 mVs^{-1} All experiments were carried out using potassium phosphate buffer (100 mM, pH = 5.8) as the electrolyte.

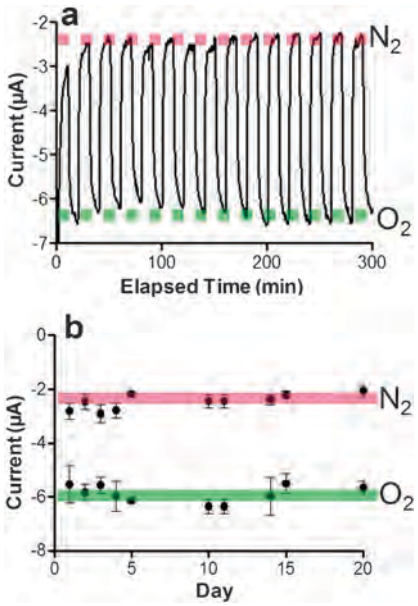

Fig. 2 Current generation from laccase-catalyzed oxygen reduction. Change in current with nitrogen- or oxygen-saturated electrolyte over sequential on–off switches (a). Stability of the minimum (N_2) and maximum (O_2) cathodic current over a period of 20 days of continuous on−off exchanges in nitrogen and oxygen-saturated electrolyte (b).

electron acceptor. Even though the introduction of CNT composites has enhanced the stability of many DET interactions, the longevity of immobilized enzymes for bioelectrocatalysis is often limited, particularly under dynamic flow conditions.[31] The stability of the laccase-functionalized CNT-BP was investigated using a stacked-cell configuration that allows electrochemical measurements of half-cell potentials under continuous flow.[32] The present electrodes maintained stable OCP of 0.622 ± 0.008 V with O_2-saturated electrolyte flowing continuously at 5 mL min^{-1}.

The potentiostatic polarization curve reveals a potential range in which the current density between N_2- and O_2-saturated electrolytes is maximal (Fig. 1b). When a selected potential (0.4 V) was applied, an abrupt change in the amperometric output arises upon transition from O_2- to N_2-saturated electrolyte (Fig. 2a). Potentiostatic measurements were selected over galvanostatic conditions because the output response was more rapid and stable.

With an applied potential of 0.4 V, the maximum (-6 μA with O_2) and minimum (-2 μA with N_2) current output of the respective states was stable and reproducible upon a repeated series of transitions from N_2- to O_2-saturated electrolyte (and *vice versa*). There was no loss in the absolute change in current ($\sim$4 μA) over $>$ 1000 cycles and a period of 20 days (Fig. 2b). Thus, the current outputs of N_2- and O_2-saturated electrolytes were assigned as an ON (O_2) or OFF (N_2) state respectively. The duration of the ON/OFF state was controlled by the pumping intervals for the flow of electrolyte.

The functional longevity of the laccase-immobilized CNT-BP confirmed that DET was sustained during continuous operation. The ON/OFF amperometric output observed with a transition from O_2- to N_2-saturated electrolyte could also be interpreted as a binary code (*i.e.*, ON = **1**, OFF = **0**). Given that a barcode can be described by a sequence of **0** and **1** values (representing white and black lines respectively), we demonstrated how the robust bioelectrocatalytic activity could generate a binary-based code sequence (ASCII 12-bit Code 39). A programmable logic controller was used to relay a sequence that varies the pumping intervals of two electrolytes and establish the means to draw the barcode lines. Specifically, turning on the pump that supplies O_2-saturated electrolyte for 10 min generated a *thin* black output line. Extending the pumping interval to 20 min produced a *thick* output line (Fig. 3). When plotted by area, the output data of current *vs.* time were visualized as a series of black or white lines. In this way, the word "TEST" was

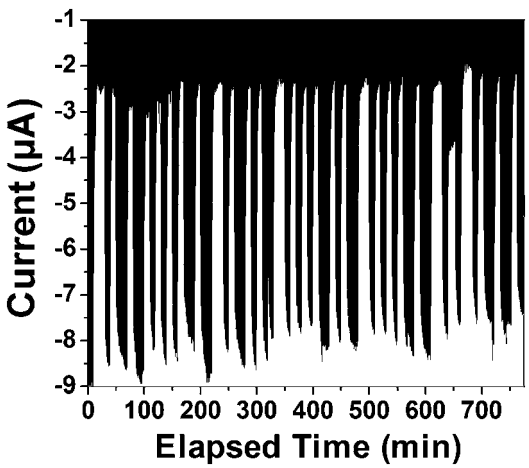

Fig. 3 Generation of a barcode from direct amperometric output of immobilized laccase. Input of N_2- or O_2-saturated electrolytes generates an amperometric output which can be programmed to yield a specific barcode by varying the residence time as demonstrated for the word "TEST". Note: The figure can be directly read using a conventional barcode scanner.

coded by varying the flow of N_2- and O_2-saturated electrolyte through the system. The resulting output data can be directly read using a conventional barcode scanner (Fig. 3).

A rapid transition in amperometric output was observed in response to changes in the chemical environment that allowed output data to be directly readable as code without any need for further treatment or processing. As the output data are not stored at the electrode, they can be reused to generate a distinct line of code; in this way, the system provides a write−rewrite function. In addition, the control sequence that defines the pumping intervals for electrolyte is not readable as a barcode (*e.g.*, 96D5A5AD 35AD2DA5 6B4AB2DA CA96D for the word "BIOCODE") until processed by the enzyme catalysis. Therefore, if the coding of the input sequence is unknown, a system of encrypted information transfer could be envisioned with potential application to cryptography.

The ability to demonstrate long-lived biocatalytic activity and retention of DET under continuous flow undoubtedly provides a substantial advance in technology development. The breadth and versatility of biomolecules and the inherent selectivity of redox enzymes offers multiple possibilities to respond to incoming signals, process them, and then relay information *ex vivo*. The biocatalyst options expand flexibility of the concept and offer significantly more complex cryptography possibilities. Moreover, the robust bio-functionalized cathode provides another step toward sustainable bio-derived energy conversion.

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